# RECONSTRUCTION EXPERIMENT OF THE TRANSDUCTION BETWEEN SINGLE H-PHASE IN SALMONELLA.

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It has been known that H-antisera inhibite in some extent the movement of the cells which have no corresponding antigen. If such inhibition happens when antisera are used for the selection of H-transduction, they may inhibite the development of the transduction swarms, which have no specific antigen corresponding them, and may cause the discrepancies of the result from what is expected. In order to test such possibilities, reconstruction experiments of the transduction between single phase cultures were performed.

MATERIALS AND METHODS.

The experimental procedures were tabulated in Table 1. SW803 b(?enx) Gal$^-$ and SW 803 (b)?enx Gal$^+$ are the same strains which have been used or obtained on the transduction between single phase donor and the mixed recipient culture of Gal-labeled single phases.

In the 2nd experiment, TM2 i(1,2) Gal$^+$, TM2 (i) 1,2 Gal$^-$, TM2 b(1,2) Gal$^+$ and TM2 (i)enx Gal$^-$ were used instead of the four strains used in the 1st experiment, and anti-i, -1,2 MGA plates were used for the selection instead of anti-b, -enx MGA plates.

RESULTS AND DISCUSSION.

The results are summarized in Table 2.

In most cases the yield of the swarm are lower than the expected, except experiment 1 where there is no significant differences between the observed and the expected. The degree of decrease may be controlled by several factors as follows:

1). Experimental error during the dilution of the cultures may cause some degree of diversity of the results. The fructuation of the loop volume from experiment to experiment suggest this possibilities. However, the strong bias

to the underestimate cannot be explained simply by the experimental error. ( When the average loop volume of the four experiments is used for the calculation, expected numbers of swarms change as in Table 3, which suggest the possibility that the experimental error is remarkable as a cause of the higher estimate in the experiment 1 rather than the lower estimate in others.)

2). The inhibitory effect of the antisera is clear on the experiment 3 and 4: the higher the concentration of antisera in a plate, the less proportions of swarms are recovered. The quantitative relationship between them is not clear from these experiments.

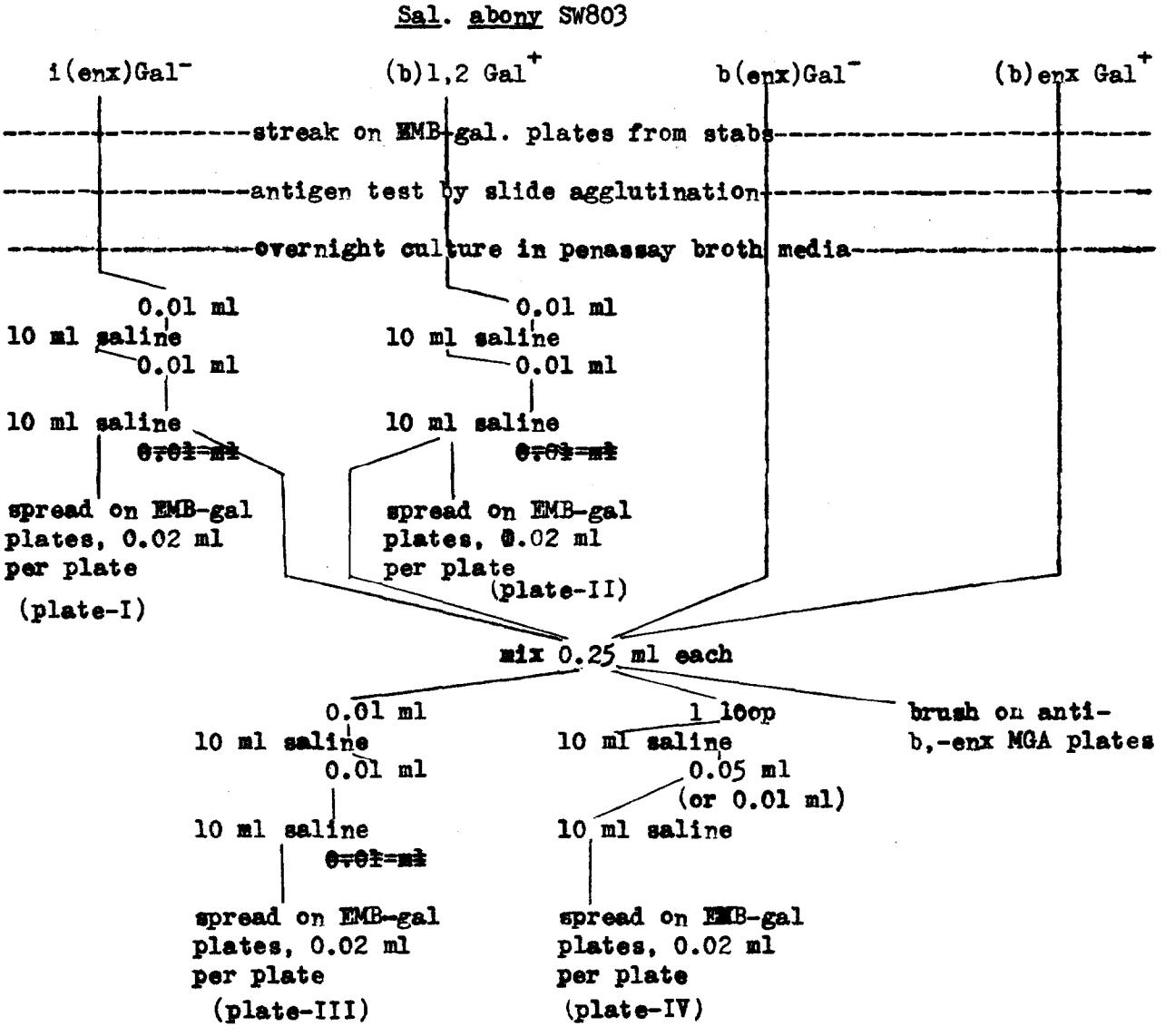
3). The condition of the media, especially the humidity of the MGA-surface, allow the real spread in various degrees. In general, the more the plate surface is humid the wider the brush spread and the recovery of swarms decrease, even if the concentration of the antisera is the same.

4). As far as the present results concern, $Gal^+$ cells decreased more than $Gal^-$ cells regardless the phase of H-antigens. However, the review of the foregoing transduction experiment does not show such regularity, so the generality of this phenomenon is still in question.

In conclusion, the method emploied for the single phase transduction experiment may results the overlook of a part of transduction in various degrees by the interference of several factors mentioned above. This loss must be considered carefully when the frequencies of the transductions are discussed quantitatively. Especially, when the transduction efficiency of the $H_1$ and $H_2$ is discussed from the ratio of $H_1$-transduced type and $H_2$-transduced type in "phase 2 --x phase 1" transduction, the third variable "the efficiency that the transducion clone can develope as swarms" must be introduced.

Table 1.

Procedures of the reconstruction experiment.




* 3 to 5 plates were used for the spread of each dilutions.

Formula to caluculate loop volume and expected number of swarms:

$$\text{Loop volume (L)} = \frac{(\text{number of colonies per plate-IV}) \times 10^{-2}}{(\text{number of colonies per plate-III}) \times 5}$$

$$\text{Expected number of swarms of "i(enx)Gal}^-\text{"} = \frac{0.25}{0.02} \times (\text{number of colonies per plate-I}) \times L$$

$$\text{Expected number of swarms of "(b)1,2Gal}^+\text{"} = \frac{0.25}{0.02} \times (\text{number of colonies per plate-II}) \times L$$

Table 2.

Results of the reconstruction experiment of H-antigen transduction between single phase cultures.

a). Experiment with TM2.

| No. of Experiment | antisera (amount per plate) | Number of colonies per plate 1 b($1_+2$)(i)enx Gal$^+$ | 2 (i)enx Gal$^-$ | 3(pipette) i($1_+2$)(i)$1_-2$ Gal$^+$ | Gal$^-$ | 4(loop) i($1_+2$)(i)$1_-2$ Gal$^+$ | Gal$^-$ | No. of swarms per brush b($1_+2$)(i)enx$^-$ Gal$^+$ | Gal$^-$ | loop volume* |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | anti-i 0.4ml anti-1,2 0.4ml | 52.0 | 36.6 | 21.0 | 16.6 | 46.0 | 41.5 | 2.8 | 2.3 | |
| | | | | | | | expected---- | 3.1 | 2.2 | $4.7\times10^{-3}$ |
| | | | | | | | % observed to expected | 90 | 105 | |
| 2 | anti-i 0.4 ml anti-1,2 0.4ml | 68.6 | 54.8 | 25.6 | 16.0 | 20.6* | 14.8* | 4.0 | 5.2 | |
| | | | | | | | expected---- | 7.3 | 5.8 | $8.5\times10^{-3}$ |
| | | | | | | | % observed to expected | 55 | 90 | |

* 0.01 ml of suspension was transfered instead of 0.05 ml on 2nd dilution

b). Experiment with SW803.

| No. of Experiment | antisera (amount per plate) | Number of colonies per plate 1 i(enx)$^-$ Gal$^+$ | 2 (b)$1,2^+$ Gal$^+$ | 3(pipette) b(enx)$^-$ Gal$^-$ | (b)enx$^+$ Gal | 4(loop) b(enx)$^-$ Gal$^-$ | (b)enx$^+$ Gal$^+$ | No. of swarms per brush i(enx)$^-$ Gal$^-$ | (b)$1,2^+$ Gal$^+$ | loop volume |
|---|---|---|---|---|---|---|---|---|---|---|
| 3 | anti-b 0.75ml anti-enx 0.4ml | 86.0 | 103.7 | 25.3 | 29.3 | 87.5 | 91.0 | 2.1 | 2.3 | |
| | | | | | | | expected----- | 7.0 | 8.4 | $6.5\times10^{-3}$ |
| | | | | | | | % observed to expected--- | 30 | 27 | |
| 4 | anti-b 0.3 ml anti-enx 0.2ml | 36.0 | 62.6 | 20.0 | 25.0 | 73.4 | 81.8 | 2.0 | 3.3 | |
| | | | | | | | expected----- | 3.1 | 5.4 | $6.9\times10^{-3}$ |
| | | | | | | | % observed to expected---- | 65 | 61 | |

Table 3.

Index of recovered % between two cultures.

| Experimental number | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Index of phase-1 to phase-2 | 0.86 | 0.61 | 1.1 | 1.6 |
| Index of Gal$^+$ to Gal$^-$ | 0.86 | 0.01 | 0.91 | 0.94 |